

AMENDMENTS TO THE CLAIMS

Claim 1. (original) A purified and isolated catalytic domain from a β (1,4)-galactosyltransferase I that catalyzes formation of galactose- β (1,4)-N-acetylglucosamine bond in the presence of magnesium.

Claim 2. (original) The catalytic domain according to claim 1, wherein the rate of formation of the galactose- β (1,4)-N-acetylglucosamine bond is at least two-fold, five-fold, ten-fold, or one hundred-fold greater than wild-type β (1,4)-galactosyltransferase I in the presence of magnesium.

Claim 3. (original) The catalytic domain according to claim 1, wherein the catalytic domain has a conservative amino acid exchange at an amino acid position corresponding to amino acid position 344 of SEQ ID NO: 6.

Claim 4. (original) The catalytic domain according to claim 3, wherein histidine is exchanged for methionine at an amino acid position corresponding to amino acid position 344 of SEQ ID NO: 6.

Claim 5. (original) The catalytic domain according to claim 1, further comprising a conservative amino acid substitution at an amino acid position corresponding to amino acid position 342 of SEQ ID NO: 6.

Claim 6. (original) The catalytic domain according to claim 5, wherein threonine is exchanged for cysteine at amino acid position 342.

Claim 7. (original) A polypeptide comprising the catalytic domain according to claim 1.

Claim 8. (original) A purified and isolated catalytic domain from a β (1,4)-galactosyltransferase I that catalyzes formation of glucose- β (1,4)-N-acetylglucosamine bond in the presence of magnesium.

Claim 9. (original) The catalytic domain according to claim 8, wherein the rate of formation of the glucose- β (1,4)-N-acetylglucosamine bond is at least two-fold, five-fold, ten-fold, or one hundred-fold greater than wild-type β (1,4)-N-galactosyltransferase I in the presence of magnesium.

Claim 10. (original) The catalytic domain according to claim 8, wherein

- (a) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 228 of SEQ ID NO : 6;
- (b) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 229 of SEQ ID NO: 6; or
- (c) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344,228, and 229 of SEQ ID NO: 6.

Claim 11. (original) The catalytic domain according to claim 10, wherein histidine is exchanged for methionine at amino acid position 344, and

- (a) lysine is exchanged for arginine at amino acid position 228,
- (b) glycine is exchanged for alanine at amino acid position 229, or
- (c) lysine is exchanged for arginine at amino acid position 228, and glycine is exchanged for alanine at amino acid position 229.

Claim 12. (original) The catalytic domain according to claim 8, further comprising a conservative amino acid substitution at an amino acid corresponding to amino acid position 342 of SEQ ID NO: 6.

Claim 13. (original) The catalytic domain according to claim 12, wherein threonine is exchanged for cysteine at amino acid position 342.

Claim 14. (original) A polypeptide comprising the catalytic domain according to claim 8.

Claim 15. (original) A purified and isolated catalytic domain from a β (1,4)-galactosyltransferase I that catalyzes formation of an N-acetylgalactosamine- β (1,4)-N-acetylglucosamine bond in the presence of magnesium.

Claims 16-21. (cancelled)

Claim 22. (currently amended) A purified and isolated catalytic domain from β (1,4)-galactosyltransferase I of claim 15 wherein the domain that catalyzes formation of an N-acetylgalactosamine- β (1,4)-glucose bond in the presence of a-lactalbumin and magnesium.

Claims 23-27. (cancelled)

Claim 28. (original) A polypeptide comprising the catalytic domain according to claim 22.

Claim 29. (original) A purified and isolated catalytic domain from a β (1,4)-galactosyltransferase I that catalyzes formation of an N-acetylglucosamine- β (1,4) -N-acetylglucosamine bond in the presence of magnesium.

Claim 30. (original) The catalytic domain of claim 29, wherein

- (a) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 228 of SEQ ID NO : 6,
- (b) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 289 of SEQ ID NO: 6, or
- (c) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344,228, and 289 of SEQ ID NO: 6.

Claim 31. (original) The catalytic domain of claim 30, wherein

- (a) lysine is exchanged for arginine at amino acid position 228,
- (b) leucine is exchanged for tyrosine at amino acid position 289, or
- (c) lysine is exchanged for arginine at amino acid position 228, and leucine is exchanged for tyrosine at amino acid position 289.

Claims 32-34. (cancelled)

Claim 35. (original) A polypeptide comprising the catalytic domain of claim 29.

Claim 36. (original) A purified and isolated catalytic domain from a β (1,4)-galactosyltransferase I that catalyzes formation of a mannose β (1,4) -N-acetylglucosamine bond in the presence of magnesium.

Claim 37. (original) The catalytic domain according to claim 36, wherein

- (a) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 228 of SEQ ID NO : 6;
- (b) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 289 of SEQ ID NO: 6; or
- (c) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344, 228, and 289 of SEQ ID NO: 6.

Claim 38. (original) The catalytic domain according to claim 37, wherein lysine is exchanged for arginine at amino acid position 228.

Claims 39-41. (cancelled)

Claim 42. (original) A polypeptide comprising the catalytic domain according to claim 36.

Claim 43. (original) A purified and isolated catalytic domain from a β (1,4)-galactosyltransferase I that catalyzes formation of a galactose- β (1,4)-N-acetylglucosamine-6-SO₃ bond in the presence of magnesium.

Claims 44-47. (cancelled)

Claim 48. (original) A polypeptide comprising the catalytic domain according to claim 43.

Claim 49. (currently amended) A nucleic acid segment encoding a catalytic domain according to claim 1 ~~any one of claims 1, 8, 15, 22, 29, 36, or 43.~~

Claim 50. (original) An expression cassette comprising the nucleic acid segment according to claim 49.

Claim 51. (currently amended) A cell comprising the nucleic acid segment according to claim 49, ~~or the expression cassette according to claim 50.~~

Claim 52. (original) A method to synthesize a galactose- β (1,4)-N-acetylglucosamine moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 1, with a donor comprising galactose, and an acceptor comprising N-acetylglucosamine.

Claim 53. (original) The method according to claim 52, wherein the donor is UDP-galactose and the acceptor is N-acetylglucosamine.

Claim 54. (original) An oligosaccharide comprising a moiety synthesized according to the method of claim 52.

Claim 55. (original) A method to synthesize a β (1,4)-N-acetylglucosamine moiety comprising:

incubating a reaction mixture comprising the catalytic domain according to claim 8, with a donor comprising glucose, and an acceptor comprising N- acetylglucosamine.

Claim 56. (original) The method according to claim 55, wherein the donor is UDP-glucose, the acceptor is N-acetylglucosamine, or the donor is UDP-glucose and the acceptor is N-acetylglucosamine.

Claim 57. (original) An oligosaccharide comprising a glucose- $\beta(1,4)$ -N-acetylglucosamine moiety synthesized according to the method of claim 55.

Claim 58. (original) A method to synthesize an N-acetylglucosamine- $\beta(1,4)$ -N-acetylglucosamine moiety comprising:

incubating a reaction mixture comprising the catalytic domain according to claim 15, with a donor comprising N-acetylgalactosamine, and an acceptor comprising N-acetylglucosamine.

Claim 59. (original) The method according to claim 58, wherein the donor is UDP-N-acetylgalactosamine, the acceptor is N-acetylglucosamine, or the donor is UDP- N-acetylgalactosamine and the acceptor is N-acetylglucosamine.

Claim 60. (original) An oligosaccharide comprising an N-acetylgalactosamine- $\beta(1,4)$ -N-acetylglucosamine moiety synthesized according to the method of claim 58.

Claim 61. (original) A method to synthesize an N-acetylgalactosamine- $\beta(1,4)$ -glucose moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 22, α -lactalbumin, a donor comprising N-acetylgalactosamine, and an acceptor comprising glucose.

Claim 62. (original) The method according to claim wherein the donor is UDP-N- acetylgalactosamine, the acceptor is glucose, or the donor is UDP-N- acetylgalactosamine and the acceptor is glucose.

Claim 63. (original) An oligosaccharide comprising an N- acetylgalactosamine- β (1,4)-glucose moiety synthesized according to the method of claim 61.

Claim 64. (original) A method to synthesize an N-acetylglucosamine- β (1,4)-N- acetylglucosamine moiety comprising incubating a reaction mixture comprising a catalytic domain according to claim 29, with a donor comprising N- acetylglucosamine, and an acceptor comprising N-acetylglucosamine.

Claim 65. (original) The method according to claim 64, wherein the donor is UDP-N- acetylglucosamine, the acceptor is N-acetylglucosamine, or the donor is UDP-N- acetylglucosamine and the acceptor is N-acetylglucosamine.

Claim 66. (original) An oligosaccharide comprising an N- acetylglucosamine- β (1,4)-N- acetylglucosamine moiety synthesized according to the method of claim 64.

Claim 67. (original) A method to synthesize a mannose β (1,4)-N- acetylglucosamine moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 36, with a donor comprising mannose, and an acceptor comprising N-acetylglucosamine.

Claim 68. (original) The method according to claim 67, wherein the donor is UDP-mannose, the acceptor is N-acetylglucosamine, or the donor is UDP-mannose and the acceptor is N-acetylglucosamine.

Claim 69. (original) An oligosaccharide comprising a mannose $\beta(1,4)$ -N-acetylglucosamine moiety synthesized according to the method of claim 67.

Claim 70. (original) A method to synthesize a galactose- $\beta(1,4)$ -N-acetylglucosamine-6-SO₃ moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 43, with a donor comprising galactose, and an acceptor comprising N-acetylglucosamine-6-SO₃.

Claim 71. (original) The method according to claim 70, wherein the donor is UDP-galactose, the acceptor is N-acetylglucosamine-6-SO₃, or the donor is UDP-galactose and the acceptor is N-acetylglucosamine-6-SO₃.

Claim 72. (original) An oligosaccharide comprising a galactose- $\beta(1,4)$ -N-acetylglucosamine-6-SO₃ moiety synthesized according to the method of claim 70.

Claim 73. (currently amended) A method comprising incubating a reaction mixture comprising an antigen having an acceptor, a donor, and the catalytic domain according to claim 8 ~~any one of claims 8, 15, 22, 29, 36, or 43~~ under conditions wherein $\beta(1,4)$ -galactosyltransferase I catalyzes bond formation between the donor and the acceptor on the antigen and causes an increase in the immunogenicity of the antigen.

Claim 74. (original) The method according to claim 73, wherein the donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, and UDP-N-acetylgalactosamine.

Claim 75. (original) The method according to claim 73, wherein the acceptor is a carbohydrate, a glycoprotein, or a glycolipid.

Claims 76-79. (cancelled)

Claim 80. (original) A method to prepare a saccharide composition having a defined sequence comprising:

contacting an acceptor with a first donor in the presence of a first catalytic domain to catalyze linkage of the acceptor with the donor to form a first saccharide composition; and

contacting the first saccharide composition with a second donor in the presence of a second catalytic domain to catalyze linkage of the first saccharide composition with the second donor to form a second saccharide composition,

wherein at least the first catalytic domain or the second catalytic domain is a catalytic domain according to claim 1-any one of claims 1, 8, 15, 22, 29, 36, or 43, and the other first or second is selected from the group consisting of a galactosyltransferase, a sialyltransferase, a fucosyltransferase, an N-acetylgalactosaminyltransferase, an N-acetylglucosaminyltransferase, and a glucuronyltransferase.

Claim 81. (original) The method according to claim 80, wherein the first donor or the second donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, UDP-N-

acetylgalactosamine, UDP-glucuronic acid, GDP-Fucose, and CMP-N-acetylneuraminic acid.

Claim 82. (original) The method according to claim 80, wherein the acceptor is a carbohydrate, a glycoprotein, or a glycolipid.

Claims 83-86. (cancelled)

Claim 87. (original) A composition prepared according to the method of claim 80.

Claim 88. (currently amended) A kit comprising packaging material, and a polypeptide comprising the catalytic domain of ~~claim 8 any one of claims 8, 15, 22, 29, 36, or 43.~~

Claim 89. (original) The kit according to claim 88, further comprising a donor.

Claim 90. (original) The kit according to claim 89, wherein the donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, UDP-N-acetylgalactosamine, UDP-glucuronic acid, GDP-Fucose, and CMP-N-acetylneuraminic acid.

Claim 91. (currently amended) A method to link a donor into an acceptor that is attached to a blood platelet comprising contacting the blood platelet with a donor and at least one catalytic domain according to ~~claim 1 any one of claims 1, 8, 15, 22, 29, 36, or 43~~ to form a reaction mixture, and incubating the reaction mixture

under conditions where the catalytic domain catalyzes linkage of the donor to the acceptor.

Claim 92. (original) The method according to claim 91 wherein the donor is exogenous UDP- galactose.

Claim 93. (original) The method according to claim 91 wherein the donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, UDP-N-acetylgalactosamine, UDP-glucuronic acid, GDP-Fucose, and CMP-N-acetylneuraminic acid.

Claim 94. (original) The method according to claim wherein the acceptor is a carbohydrate, a glycoprotein, or a glycolipid.

Claim 95. (original) The method according to claim wherein the acceptor is N- acetylglucosamine.